

The influence of predator odours and overcrowded mouse odours on regulation of oestrous cycles in house mice (*Mus musculus*)

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Abstract. We investigated the influence of chemical signals derived from different sources—urine of feral cats (*Felis catus*) and urine from overcrowded mice (*Mus musculus*) on regulation of oestrous cycles in *Mus musculus musculus* under laboratory conditions.

Cat urine and urine from mice housed in overcrowded conditions caused very similar effects. Application of urine from feral cats and from overcrowded conspecifics to the bedding of experimental animals for a period of 21 days caused a significant increase in numbers of animals with extended oestrous cycles. Application of cat urine or overcrowded mouse urine to the bedding of female mice caused an extension of oestrous cycles in 56.0% and 62.5% of tested animals, respectively.

The results of the present study and other experimental data from our laboratory may indicate that predator urine and urine from overcrowded conspecifics share the same chemical information.

Introduction

Mammals are known to avoid predator odours (Muller-Schwarze 1983; Weldon 1990; Ylonen et al. 1992; Eppli et al. 1993; Jedrzejewski et al. 1993). More than 10 years ago, the presence of predator urine was shown to decrease activity patterns in rodents (Sullivan et al. 1988). The mechanism of repellency is putatively a fear-induced avoidance response by rodents (potential prey) to the urine characteristics of predators on meat diets, i.e. the double-bonded sulfur and amino by-products of protein digestion (Nolte et al. 1994). In our investigations of the effects of predator urine on rodent behaviour, we found that exposure of rats (*Rattus norvegicus*), mice (*Mus musculus*), and voles (*Clethrionomys rutilus*) to predator urine significantly reduced the reproductive output of these rodents. Previously, we demonstrated that oestrous cycles were extended in Norway rats exposed to mink (*Mustela vison*) anal sac secretions (Voznessenskaya et al. 1992) and urine of feral cats (Feoktistova et al. 2001). Similarly, Kostela et al. (1996) found there was an extension of the duration of oestrous cycles in bank voles (*Clethrionomys glareolus*) exposed to weasel (*Mustela nivalis*) odours.

If food becomes limiting, rodents will begin catabolising their muscle protein and so their urine would contain larger amounts of protein digestion products (Swick and Benevenga 1977; Baracos et al. 1983). These

signals could serve to trigger mechanisms that would curtail reproduction.

In the present work, we compare the effects of feral cat urine and urine from mice housed in overcrowded conditions on the regulation of oestrous cycles in house mice under laboratory conditions.

Materials and methods

Test subjects were 36 mice of 4–6 months of age from an outbred laboratory population. Before the experiments, females were housed singly in standard plastic cages. Each cage was placed in individual box (size 70 × 20 × 35 cm) with a separate ventilation system for every animal. Air-flow from different cages did not mix at any stage of the experiment.

Experimental rooms were illuminated on 12:12 hours light:dark schedule, and maintained at 20–22°C. Food and tap water were provided *ad libitum*.

Oestrous cycles were quantified by taking vaginal smears for 21 days before treatments (3–4 complete cycles). After the different types of treatments, oestrous cycles were monitored for a further 21 days. We used two basic treatments: (1) urine from feral cats (*Felis catus*) and (2) urine from overcrowded mice (*Mus musculus*). For the control group of animals (water treatment), we monitored oestrous cycles for the duration of the experiment (42 days).

Urine from feral cats (*F. catus*) was used as a source of predator chemical cues. The cats used in the experiment normally hunt for mice and have mice in their diet. If needed, additional meat was added to their diet. Freshly voided urine was frozen (22°C). Once defrosted, urine was used only 1–2 days. Non-predator urine was obtained from house mice (*M. musculus*). Individuals ($n = 12$) of both sexes were placed in a standard cage for 14 days before urine collection. Food and water were available *ad libitum*. Mice were placed into metabolic stainless steel cages, overnight, and urine was collected and stored using the method described above.

Urine (0.5 mL) (corresponding to specific treatment) or water was put directly onto the bedding of female mice every day for the duration of the treatment. For the first group ($n = 12$) water only was used. For the second group ($n = 12$) water was applied for the first 21 days, then cat urine was applied for the next 21 days. For the third group ($n = 12$) water was applied for the first 21 days, then urine from overcrowded conspecifics was applied for the remaining 21 days. The number of females with extended oestrous cycles relative to both of the controls was recorded. Also we recorded the number of times animals were in oestrus for each group for the period of manipulation (21 days).

Differences among treatment groups were determined using the *t*-test (Student criterion) and Fisher test (STATISTICA, StatSoft Inc., Tulsa, Oklahoma, 1999).

Results and discussion

Cat urine applied every day to the bedding of female mice for a period of 21 days caused a significant ($p < 0.001$) increase in the numbers of animals with extended oestrous cycles relative to control animals (Table 1). Urine from overcrowded conspecifics applied to the bedding of female mice on a daily basis had even greater effect. In this case, 62.5% of female mice had extended cycles relative to the control animals. For both treatments, the duration of the delay to the next oestrus ranged from 2 to 14 days. The average number of oestruses is shown in Table 1. We observed regular cycles for all animals in control group. The number of oestruses was significantly reduced ($p < 0.001$) in animals exposed to the cat urine

and in the group treated with urine from overcrowded conspecifics ($p < 0.001$).

Conclusions

The results of the present study suggest that predator (feral cat) urine and urine from overcrowded mice may share similar information about unfavourable conditions for reproduction. The responses are similar to those reported in previous studies on rats, mice and voles (Feoktistova et al. 2001; Voznessenskaya 2002; Voznessenskaya et al., this volume). Mice respond to chemical cues of different origin in the similar manner: reducing numbers of cycling animals and lowering the probability of pregnancy.

The chemical cues of cat urine and urine from mice housed in overcrowded conditions may be due to each containing some similar chemical components as a result of protein digestion in carnivores and muscle catabolism in overcrowded rodents (e.g. sulfur-containing compounds, amino acids, and peptides) (Nolte et al. 1994).

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References

- Eppli, G., Mason, J.R., Nolte, D.L. and Campbell, D.L. 1993. Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). *Journal of Mammology*, 74, 715–722.
- Baracos, V., Rodemann H.P., Dinarello, C.A. and Goldberg, A.L. 1983. Stimulation of muscle protein degradation and prostaglandin E₂ release by leukocytic pyrogen (interleukin-1). *The New England Journal of Medicine*, 308, 553–558.
- Feoktistova, N.Yu., Naidenko, S.V., Kartashova, E.A. and Voznessenskaya, V.V. 2001. The role of predator odors in regulation of estrous cycles in small rodents. Supplement to *Ethology. Contributions to the XXVII International Ethological Conference*, p. 152.

Table 1. The influence of cat urine and urine derived from overcrowded conspecifics on the number of periods of oestrus during the treatment period in *Mus musculus*.

Treatment	Number of periods of oestrus pre-treatment (21 days)	Number of periods of oestrus during treatment (21 days)	Percentage of animals with extended oestrous cycles
Control (water only)	4.2 ± 0.1	4.3 ± 0.2	0
Cat urine	4.3 ± 0.2	3.0 ± 0.2	56.0
Overcrowded mouse urine	4.8 ± 0.3	3.0 ± 0.3	62.5

- Jedrzejewski, W., Rychlik, L. and Jedrzejewski, B. 1993. Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). *Journal of Mammology*, 74, 715-722.
- Kostela, E., Horne, T., Mappes, T. and Ylonen, H. 1996. Does risk of small mustelid predation affect the oestrous cycle in the bank vole, *Clethrionomys glareolus*? *Animal Behaviour*, 51, 1159-1163.
- Muller-Schwarze, D. 1983. Experimental modulation of behavior of free-ranging mammals by semiochemicals. In: Muller-Schwarze, D. and Silverstein, R.H., ed., *Chemical signals in vertebrates 3*. New York, Plenum Press, p. 368.
- Nolte, D.L., Mason, J.R., Eppie, G., Aronov, E. and Campbell, D.L. 1994. Why are predator urines aversive to prey? *Journal of Chemical Ecology*, 20, 1505-1516.
- Sullivan, T.P., Grump, D.R. and Sullivan D.S. 1988. The use of predator odor as repellents to reduce feeding damage by herbivores. IV. Northern pocket gophers (*Thomomys talpoides*). *Journal of Chemical Ecology*, 4, 379-389.
- Swick, R.W. and Benevenga, N.J. Labile protein reserves and protein turnover. 1977. *Journal of Dairy Science*, 60, 505-515.
- Voznessenskaya, V.V. 2002. Predator urine as a reproductive inhibitor for rats. *Journal of Chemical Ecology* (in press).
- Voznessenskaya, V.V., Wysocki, C.J. and Zinkevich, E.P. 1992. Regulation of the rat estrous cycle by predator odors: role of the vomeronasal organ. In: Doty, R.L. and Muller-Schwarze, D., ed., *Chemical signals in vertebrates*. New York, Plenum Press, 281-284.
- Weldon, P. 1990. Responses by vertebrates to chemicals from predators. In: MacDonald, D.W., Muller-Schwarze, D. and Natynczuk, S.E., ed., *Chemical signals in vertebrates*. New York, Oxford University Press, 500-521.
- Ylonen, H., Jedrzejewska, B., Jedrzejewski, W. and Heikkila, J. 1992. Antipredatory behaviour of *Clethrionomys voles*—“David and Goliath” arms race. *Annals of Zoology Fennoscandia*, 29, 207-216.

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